FORESTRY

FRDA REPORT 144

INFLUENCE OF SOIL TEMPERATURE ON THE EFFECTIVENESS OF ECTOMYCORRHIZAL FUNGI IN PROMOTING WHITE SPRUCE SEEDLING GROWTH

by
L.D. Husted

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by
L.D. Husted

Contractor: Faculty of Forestry
University of British Columbia
Vancouver, B.C.

Departmental Representative:

Dr. J.A. Trofymow
Pacific Forestry Centre
Forestry Canada
506 W. Burnside Road
Victoria, B.C.

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ABSTRACT

The purpose of this FRDA project was to study the influence of soil temperature on the effectiveness of various ectomycorrhizal (ECM) fungi i.e., their ability: (1) to colonize roots a nursery, (2) to persist and colonize new roots in the field and (3) to improve the growth and physiology of white spruce (Picea glauca (Moench) Moss) seedlings.

The early growth of planted white spruce seedlings is less than satisfactory on many backlog reforestation sites in the northern interior of British Columbia. Artificial inoculation of conifer seedlings with specific ECM fungi has been shown to improve field performance on some reforestation sites. However, it was not known if inoculation would benefit seedlings planted in cool forest soils. Research with vesicular-arbuscular mycorrhizae has shown that cool temperatures eliminate the benefits normally derived from mycorrhizal infection

Soil temperature influenced all aspects of ECM effectiveness. A better understanding of the relationships between environmental parameters, such as temperature, and mycorrhizal effectiveness will help unravel the contradictory results of artificial inoculation studies. The study results have two important implications for the reforestation of cool sites: (1) artificial inoculation of spruce seedlings, with forest soil inoculum or with specific ECM fungi, has good potential to improve seedling growth and nutrient uptake in the first year after outplanting and (2) infection with some ECM fungal isolates (e.g., E-strain and H. crustuliniforme in this study) may reduce seedling nutrient uptake and growth in the first year after planting. It

would be useful to know the effectiveness of fungi which naturally colonize spruce seedling roots in container and bareroot nurseries.

Forest soil from a vigorous spruce plantation was an effective source of inoculum. Seedlings inoculated with forest soil may benefit from the greater diversity of ECM fungal propagules and rhizosphere organisms contained in soil inoculum compared to pure culture inoculum. Research should be directed to examining the effects of the planting site environment and forest management practices (e.g., mechanical site preparation) on indigenous ECM fungal propagules in order to identify sites and silvicultural regimes most likely to benefit from artificial inoculation of nursery seedlings.

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INTRODUCTION

The survival and growth of planted white spruce (Picea glauca (Moench) Moss) seedlings is less than satisfactory on backlog reforestation sites in the northern interior of British Columbia [15]. Planting check, a period of slow shoot growth after planting, is common and results in reduced growth (50% of normal) for several years after planting. This increases the susceptibility of spruce seedlings to brush competition.

The degree to which nursery seedlings are preconditioned to the planting site has a great influence on seedling field performance [13]. Preconditioning seedlings, by inoculating them with specific ectomycorrhizal (ECM) fungi adapted to the planting site, has been shown to improve field performance [17] [49] [50] [58] [66] [98]. Mycorrhizal seedlings may benefit from enhanced nutrient or water uptake, increased resistance to pathogens or slower root turnover [38].

However, mycorrhizal inoculation does not always improve seedling performance [10] [85]. The potential benefits of a nursery inoculation program are greatest when the growth of outplanted seedlings is limited by water or nutrient availability in the first growing season, the inoculum potential of indigenous fungi at the planting site is low, and the inoculant fungus is adapted to the planting site environment.

Mycorrhizal inoculation of spruce seedlings has good potential for improving the reforestation of white spruce on backlog sites for three reasons:

(1) Planting check of white spruce appears to result from poor root growth, water and nutrient uptake in the first growing season [14] [15].

(2) The inoculum potential of backlog sites may be low. Danielson [22] assessed mycorrhizal colonization on bare-root spruce planted in a site preparation trial near Prince George. New roots formed outside the original root mass were not colonized by indigenous fungi in the first growing season. He concluded that the inoculum potential of backlog sites may be lower and less predictable compared with that of recently logged sites or more southern reforestation sites where most mycorrhizal research has been conducted.

(3) Seventy percent of interior spruce seedlings are grown in southern nurseries. ECM fungi colonizing seedlings in warm (15 to 35°C), fertile container growing mixes may not be adapted to the cool (5 to 10°C), less fertile soils of northern planting sites.

The overall objective of FRDA 52-41-109 was to study the influence of soil temperature on the effectiveness of ECM fungi. The effectiveness of a particular ECM fungus will depend on (1) its aggressiveness in conifer nurseries (degree of root colonization), (2) its persistence in the field and (3) its inherent ability to promote seedling growth [94].

Although it is known that soil temperature alters the growth of ECM fungi in culture [28] [37] [60] [82] [91] and in forest soils [40] [55] [70], the influence of soil temperature on the effectiveness of ECM fungi, in particular their ability to promote seedling growth, is poorly understood [72].

Research with vesicular-arbuscular mycorrhizae (VAM) has shown that cool temperatures eliminate the benefits normally derived from mycorrhizal infection [65] [87]. The growth of VAM plants may even be reduced compared to that of nonmycorrhizal plants at low temperatures [32] [39] [84].

Results for each FRDA 52-41-109 experiment are presented separately. However, all experiments are summarized together in the final discussion of the report (page 35). Five experiments were conducted:

(1) Root-zone temperature and ectomycorrhizae formation.

(2) Cool soil temperatures and persistence of established ectomycorrhizae.

(3) Effect of inoculation on the physiology of cold-stored seedlings immediately after planting into cold soils.

(4) Interaction of soil temperature and inoculation on the growth and nutrition of spruce seedlings.

(5) Effect of slow-release fertilizer at the time of planting on the persistence of established mycorrhizae.

SECTION ONE

OBJECTIVE

Determine the effect of three growing mix temperatures, 6, 16, and 26oC on mycorrhiza formation by a variety of fungal isolates collected from field and container nursery environments. It was hypothesized that isolates collected from nursery environments would form mycorrhiza more rapidly at 26°C and more slowly at 6°C compared to isolates collected from forest sites.

BACKGROUND INFORMATION

The rate at which mycorrhiza colonize root systems affects the ability of mycorrhiza to enhance host plant growth and physiology. The most effective fungi are often those that rapidly and extensively colonize seedling root systems [1] [72].

Soil temperature influences the growth of ECM fungi in pure culture [28] [37] [60] [82] [91] and the rate of ectomycorrhizae formation when seedlings are planted in forest soil [40] [55] [70]. These studies suggest that the optimum temperature for fungal growth in culture and ectomycorrhiza formation in forest soils lies between 18 and 26°C, i.e., temperatures that are well above the normal soil temperatures for northern planting sites. However, significant intra-and interspecific variation in fungal response to temperature in culture experiments has been reported [28] [82] with some fungal isolates more tolerant of temperature extremes than others.

METHODS

Surface-sterilized white spruce seed (SL# 29144) were sown in 40 cm^3 Spencer-LeMaire Rootrainers filled with a pasteurized peat:vermiculite growing

mix used at the Forestry Canada (Victoria B.C.) container nursery: 110 l (1 bale) of compressed Premier brand 'Forestry Peat', 57 l (2 cu. ft) medium grade horticulture vermiculite, 175 g Micromax Micronutrients (Sierra Chemical Co., Milpitas, CA) and 750 g coarse dolomite lime. Seedlings were grown in a controlled environment chamber (18 hr photoperiod, 20°C air temperature, 70-90% relative humidity).

Eight weeks after germination, the seedlings were inoculated with either:

a) 4 cm^3 of a mycelial slurry prepared from one of twelve pure cultures of ECM fungi, or

b) 4 cm^3 of screened forest floor from northern B.C., or

c) 4 cm^3 of autoclaved agar culture medium (control treatment).

The twelve pure culture ECM isolates are listed in Table 1. Five were collected from container nurseries; eight from field sites (Appendix A details the source of each isolate).

After inoculation, the seedlings were grown at either a 6, 16 or 26°C root-zone temperature in a controlled environment chamber (18 hr photoperiod, 16-22°C air temperature, 30-70% relative humidity, light intensity of 400 uE $m^{-2}s^{-1}$) at the University of British Columbia. Water baths were used to maintain the growing mix temperatures. Seedlings were watered as necessary to maintain the growing mix water potential above field capacity. Once a week, 10 ml of a water-soluble fertilizer solution (100 ppm N) containing micronutrients (Plant-Prod 20-20-20) was added to each seedling container.

Eight weeks after inoculation, mycorrhiza formation was assessed by scanning the whole plug at 12-40x magnification and checking whole mounts at 1000x magnification [24]. Mycorrhiza formation was recorded on a six-class scale: (1) 0% of short roots mycorrhizal, (2) 1-25% mycorrhizal, (3) 26-50%

mycorrhizal, (4) 51-75% mycorrhizal, (5) 76-95% mycorrhizal, (6) > 95% mycorrhizal. A short root was considered mycorrhizal if a Hartig net was present.

The experiment was a completely randomized design with a factorial (3 x 14) arrangement of the treatments; there were 12 seedlings planted per treatment combination. The resulting data were not normally distributed and in some treatment combinations there was zero variation. Therefore, a non-parametric randomization test [86] with a subsample of 100 000 random permutations [29] was used to test differences among inoculation and temperature treatments. Interactions between soil temperature and inoculation treatments were examined graphically. Mean mycorrhiza class for each inoculation and root-zone temperature treatment combination was used in a two-way analysis of variance (ANOVA) to test the effect of inoculum source (field or nursery) and root-zone temperature on mycorrhiza formation.

RESULTS

Interaction of temperature and inoculation treatments

The effect of inoculum source and soil temperature on mean mycorrhiza class is shown in Table 1. Graphs of these data indicated that there were important interactions between temperature and inoculation treatments. Mycorrhiza formation increased with temperature with the E-strain 947, *T. terrestris* 2088, *T. terrestris* Laval and *L. laccata* 101C inocula; decreased with temperature for *L. bicolor* 5313; was greatest at 16°C and lower at both 6°C and 26°C for the other nine inocula.

Table 1. Effect of inoculum and root-zone temperature on mean mycorrhiza formation (n=12, standard deviation in brackets).

Inoculum	Root-zone temperature (oC)		
	6	16	26
Control	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Amphinema byssoides	1.1 (0.3)	1.7 (0.7)	1.3 (0.5)
E-strain 0188	1.3 (0.5)	2.6 (1.5)	2.2 (1.2)
E-strain 947	2.8 (1.3)	5.3 (0.9)	6.0 (0.0)
Forest floor	3.4 (1.7)	5.0 (1.1)	2.7 (1.3)
Hebeloma crustuliniforme 5	1.0 (0.0)	2.5 (1.6)	1.3 (0.5)
H. crustuliniforme 8	1.0 (0.0)	1.8 (1.0)	1.5 (0.5)
H. crustuliniforme 5247	1.0 (0.0)	2.3 (1.1)	1.8 (0.8)
H. crustuliniforme 125	2.2 (0.7)	2.7 (0.9)	2.3 (0.5)
Laccaria bicolor 5313	2.8 (1.3)	2.7 (1.4)	1.2 (0.4)
L. bicolor 5268	4.7 (0.5)	5.4 (0.5)	4.3 (1.1)
L. laccata 101C	1.5 (0.5)	3.2 (1.7)	3.9 (1.0)
Thelephora terrestris 2088	1.0 (0.0)	4.8 (0.8)	5.9 (0.3)
T. terrestris Laval	1.0 (0.0)	1.6 (1.2)	3.0 (2.3)

Effect of soil temperature

Averaged across all inoculation treatments, mycorrhiza formation was lower at 6°C ($p < 0.001$) and 26°C ($p = 0.15$) compared with that at 16°C. Mean mycorrhiza classes for the 6, 16 and 26 °C root-zone temperatures were 1.9, 3.0, and 2.8, respectively. Poor mycorrhiza formation at low soil temperatures can result from weak fungal growth or from limited root growth, and therefore, fewer available colonization sites for ECM fungi [69]. In this experiment, it likely resulted from weak fungal growth. The seedlings were grown for eight weeks in a warm growing medium (18°C) before inoculation to ensure that there were many short roots (>100/seedling) available for colonization.

Effect of inoculum and inoculum source

Mean mycorrhiza class for each inoculation treatment was averaged across all root-zone temperatures (Table 2). Two nursery isolates (*L. laccata* 101C and *T. terrestris* 2088) and three field sources of inoculum (forest floor, *L. bicolor* 5268 and E-strain 947) formed the most mycorrhiza eight weeks after inoculation.

ANOVA showed no effect ($p = 0.50$) of inoculum source (field or nursery) on mycorrhizal colonization. No interaction occurred between temperature and inoculum source ($p = 0.87$).

Table 2. Mean mycorrhiza class by inoculation treatment (n = 36 seedlings).

Inoculation treatment	Inoculum source*	Mean mycorrhiza class	
Control	-	1.0	
Hebeloma crustuliniforme (8)	F	1.4	b**
Amphinema byssoides (0288)	N	1.4	b
Hebeloma crustuliniforme (5)	F	1.4	b
Hebeloma crustuliniforme (5247)	F	1.7	bc
Thelephora terrestris (Laval)	F	1.9	bc
Laccaria bicolor (5313)	N	2.1	cd
E-strain (0188)	N	2.2	cd
Hebeloma crustuliniforme (125)	F	2.4	cd
Laccaria laccata (101C)	N	2.8	de
Forest floor	F	3.5	ef
Thelephora terrestris (2088)	N	3.9	fg
E-strain (947)	F	4.5	gh
Laccaria bicolor (5268)	F	4.8	h

* F = field source; N = nursery source.

** Means followed by the same letter are not significantly different ($p = 0.05$).

Temperature effects on mycorrhiza formation have at least two implications for artificial inoculation in forest regeneration programs:

(1) Will it be possible to successfully inoculate seedlings growing in warm container nurseries with fungal isolates indigenous to cool soil environments?

(2) Will isolates adapted to a warm nursery environment be able to colonize roots formed after the seedlings are planted into cool soils?

Based on the fungal isolates used in this study, the answer to both question is "yes". Although there were differences among isolates in mycorrhiza formation at the various soil temperatures, these differences were not related to the collection site (field or nursery). Some field isolates formed mycorrhiza well at 26°C; some nursery ones formed mycorrhiza relatively well at 6°C. The number of test fungi is too small to extend the results beyond these specific isolates. However, Samson and Fortin [82] reported that the growth of 62 fungal isolates in culture was not related to the geographic origin of the isolate.

Growing mix temperature likely plays an important role in mycorrhiza formation and competition between inoculant fungi and indigenous nursery fungi in container nurseries. T. terrestris, a common indigenous nursery fungus, which often contaminates artificial inoculation experiments, had a higher rate of mycorrhiza formation at 26°C compared to many of the other isolates. This could give it an advantage in container nursery medium.

Mycorrhiza formation occurred within eight weeks after inoculation at the 6°C mix temperature. Heninger and White [40] also grew spruce seedlings in forest soil for eight weeks at various soil temperatures ranging from 15 to 31°C. In contrast to this study, they found no mycorrhiza formation at soil

temperatures below 19°C. The different results of the two studies probably reflect the method of mycorrhiza assessment. Heninger and White [40] did not use high power magnification to check for initial stages of infection such as Hartig nets; short roots were assessed "visually" for presence or absence of mantles.

SECTION TWO

OBJECTIVE

Study the ability of mycorrhizal fungi established in the nursery to persist in the original root plug and to colonize roots formed outside the plug after seedlings are transplanted into cool soils.

BACKGROUND INFORMATION

High soil temperatures (35°C for one week) have been shown to reduce the persistence of mycorrhizae [70]. The effect of cold soil temperatures on persistence and new root colonization has not been studied.

Persistence of inoculant fungi varies from one to four years [10] [26] [62] [93]. Competition from indigenous forest mycorrhizal fungi is often assumed to be the major factor affecting persistence [63] although the dynamics of field persistence have not been thoroughly researched. Danielson and Visser [26] found that inoculant fungi failed to persist and colonize new roots even when indigenous competition was weak. They concluded that poor adaptation of the inoculant fungi to the physical, chemical or non-mycorrhizal biological components of the planting site may be more important than competition from indigenous fungi in determining persistence.

Persistence is also related to the degree of root colonization by the inoculant fungus at the time of planting. Inoculum density is a major factor determining the ability of a fungal isolate to form mycorrhiza. Analysis of data presented by [26] for nine ECM fungi showed that percent colonization at the time of planting was positively correlated ($p = 0.006$) with percent colonization of new roots a year after planting.

METHODS

Eight-week-old spruce seedlings (SL# 29144) were inoculated, grown for ten weeks, cold-stored ten weeks at 2-3°C, and then transplanted into soils at two temperatures (6, 12°C). Five inoculation treatments were planted in forest soil with and without indigenous ECM fungal propagules at both soil temperatures (Table 3). Because of space limitations in the water baths, three additional inoculation treatments were planted only in forest soil with ECM fungal propagules.

Mycorrhiza class (as described on page 5) of each seedling was estimated at the time of transplanting and twelve weeks later using 1000x magnification. Roots within the original container plug and new roots formed outside the plug were assessed separately for the second sample. Data were subjected to a paired-sample randomization test [86]; to compensate for differences in mycorrhizal colonization at the start of the experiment, persistence on new roots was compared to persistence on roots within the original plug.

Table 3. Inoculation treatments - Experiment Two.

Forest soil* with indigenous ECM fungi	Forest soil without indigenous ECM fungi**
Uninoculated (control)	control
E-strain (947)	E-strain (947)
Hebeloma crustuliniforme (5249)	Hebeloma crustuliniforme (5249)
Laccaria laccata (101D)	Laccaria laccata (101D)
Forest floor	Forest floor
Hebeloma crustuliniforme (125)	Hebeloma crustuliniforme (125)
Laccaria bicolor (5268)	
Amphinema byssoides (0288)	
Thelephora terrestris (0288)	

* A mixture of forest floor, mineral soil and perlite (1:1:2 by volume). The forest floor and mineral soil were collected from 3 spruce plantations near Mackenzie, B.C.

** The forest floor mixture was pasteurized (65°C, 30 min) to kill indigenous fungal propagules and then inoculated with soil microbes extracted from unpasteurized forest floor.

RESULTS

Initial mycorrhiza class

Mean mycorrhiza class for the various inoculation treatments ranged from 4.2 to 6.0 at the time of transplanting (Table 4). Mycorrhizal formation was lower ($p < 0.001$) in the A. byssoides and T. terrestris treatments (mean mycorrhiza classes of 4.3 and 4.7, respectively) compared to the other isolates (mean classes 5.9 to 6.0). There was a light infection of T. terrestris (Hartig net present on less than 25% of the short roots) of some seedlings inoculated with A. byssoides.

Persistence within original root plug

Persistence of the inoculant fungi was high within the original root plug. Mean mycorrhiza class ranged from 4.7 to 6.0. Competition from indigenous fungi and soil temperature had no effect on persistence within the original root plug.

Colonization of roots formed outside the container plug

Overall, colonization of these roots by inoculant fungi was high (> 75%) even though the potential for mycorrhiza formation by indigenous ECM fungi was significant. The root systems of uninoculated seedling transplanted into the unpasteurized soil mixture used in this experiment were rapidly colonized by indigenous forest ECM fungi. At five weeks, 66% of these seedlings had some mycorrhiza formation; at twelve weeks, 100% and 95% of the seedlings, respectively in the 6 and 12°C soil temperatures, had considerable mycorrhiza formation (greater than 37% and 50% respectively).

Five inoculation treatments were planted with and without competition at 6 and 12°C soil temperatures. There was an interaction between competition from indigenous ECM fungi, soil temperature and inoculation treatments on colonization of new roots. Competition reduced persistence of H. crustuliniforme 5249 and L. laccata but did not affect persistence of the other inocula (Table 4). Competition reduced H. crustuliniforme 5249 persistence at both soil temperatures; but only reduced L. laccata persistence at the 12°C soil temperature. Mean mycorrhiza class for this isolate was 6.0 and 4.3, respectively, for the 6 and 12°C soil temperatures.

Eight inoculation treatments were planted at both soil temperatures in forest soil containing indigenous fungal populations. Overall, persistence was lower at 12°C than at 6°C. At 6°C, only one fungal isolate, H. crustuliniforme 5249, had lower ($p = 0.03$) persistence on new roots compared to old roots; at 12°C, three isolates, L. laccata ($p = 0.001$), H. crustuliniforme 5249 ($p = 0.06$), and A. byssoides ($p = 0.06$) had lower persistence on new roots. Indigenous forest fungi colonized short roots not occupied by L. laccata or A. byssoides. Most short roots not colonized by H. crustuliniforme 5249 remained nonmycorrhizal.

Two factors may be responsible for the lower persistence of established ECM fungi at 12°C compared to 6°C soil temperatures. Root growth increases with soil temperature resulting in a greater likelihood of short roots contacting indigenous sources of inocula at 12°C. Mean seedling root length increased by 22% and 65%, respectively for the 6°C and 12°C soils during the experiment.

Established ECM fungi grow along roots as they elongate and infect new short roots as they develop. At warmer soil temperatures they may not grow rapidly enough to reach newly-formed short roots before these roots contact indigenous ECM inocula. At low rates of root growth, established ECM fungi may be able to reach new short roots before they are colonized by indigenous ECM fungi; established fungi have the advantage of a supply of host seedling carbohydrates.

Table 4. **Mean mycorrhiza class for initial root infection and infection of new roots formed outside the container plug (n=12).**

	Initial infection		New root infection			
Competition	--		no		yes	
Temperature	6°C	12°C	6°C	12°C	6°C	12°C
E-strain	6.0	6.0	6.0	6.0	5.9	6.0
H. crustuliniforme 5249	5.9	5.8	5.7	6.0	5.4	5.3
H. crustuliniforme 125	6.0	6.0	6.0	6.0	6.0	6.0
L. laccata	6.0	6.0	6.0	6.0	6.0	4.3
Forest floor	6.0	6.0	6.0	6.0	6.0	6.0
L. bicolor	6.0	6.0	---	---	6.0	6.0
A. byssoides	4.4	4.2	---	---	4.3	3.8
T. terrestris	4.3	5.0	---	---	4.2	4.8

SECTION THREE

OBJECTIVE

Evaluate the effects of inoculation on the resistance to water uptake, pre-dawn stomatal conductance, and net photosynthesis of cold-stored spruce seedlings immediately after transplanting them into cold soils.

BACKGROUND INFORMATION

Transplant shock may result from the inability of cold-stored seedlings to quickly acclimate to the environment of the planting site [35]. Water stress can develop in seedlings transplanted into cold soils even when these soils are moist because water uptake is reduced considerably by cold soil temperatures.

Root resistance to water flow increases in cold soils, mainly due to changes in the permeability of root cell membranes and increases in the viscosity of water [46]. The high root resistance which develops in cold soils decreases seedling water absorption [80]. If seedlings transpire more water than they are able to absorb, water stress develops, resulting in poor root growth [54], lower rates of photosynthesis [27], and decreased production of carbohydrate for new shoot and root growth.

The development of severe water stress is prevented in the short-term by stomatal closure [54]. However, cold-stored spruce seedlings may not be able to regulate their stomata immediately after transplanting. Grossnickle and Blake [35] found that spruce stomata in contrast to those of pine seedlings did not fully close at night for approximately two to three weeks after the seedlings were removed from cold storage.

METHODS

Eight-week-old seedlings in 40 cm^3 Spencer-LeMaire Rootrainers were inoculated and grown (as described on page 11), then transplanted into a mixture of forest floor, mineral soil and perlite (1:1:2 by volume) maintained at either 6°C or 12°C.

Six inoculation treatments were included in this experiment: (1) 4 cm^3 forest floor from vigorous spruce plantations, (2) 4 cm^3 of sterilized agar (control), or 4 cm^3 of a mycelial slurry prepared from pure cultures of (3) Hebeloma crustuliniforme 5249, (4) Laccaria bicolor 5268, (5) E-strain 947, and (6) Thelephora terrestris (Laval). All of the pure culture fungi were collected from forest sites (Appendix A). Mycorrhizal colonization by inoculant and nursery contaminant fungi was assessed (1000x magnification) at the time of transplanting (results are summarized in Appendix B).

The seedlings were not fertilized after transplanting but were watered with tap water to maintain soil moisture tension at $-10 Jkg^{-1}$ (0.1 bar) i.e., the soils were both cool and moist. The three week study was conducted in a controlled environment chamber (18 hr photoperiod, relative humidity of 30 to 50%, air temperature of 12 to 20°C, and a light intensity of 400 uE $m^{-2}s^{-1}$.)

At the start of the experiment, two bulked samples of root and shoot tissue (eight seedlings per sample) from each inoculation treatment were analysed for nitrogen (N) and phosphorus (P) concentrations (oven-dry mass basis). After oven-drying (70°C, 48 hours), milling and wet oxidation with a Caro's acid digest [71], tissues were analyzed for N and P by colorimetric (auto analyser) technique (salicylate/nitroprusside for N; ascorbic acid/molybdate-antimony for P).

Seedling caliper, root parameters (biomass, lateral root length, number of short roots), pre-dawn stomatal conductance and mid-day (4 hours into the light period) net photosynthesis were measured 2, 9 and 21 days after transplanting. Root biomass was determined after oven-drying at 70°C for 48 hours. Stomatal conductance and net photosynthesis were measured using a portable LiCor (6200) gas exchange system. All measurements were made on mature needles; newly flushed needles were removed carefully prior to measurement. Net photosynthetic rates were calculated on the basis of needle surface area estimated from needle length and displaced volume [11].

Root resistance to water uptake was estimated by calculating R_{SPAC} (resistance to water flow through the soil-plant-atmosphere continuum). Root resistance is the major resistance along this pathway unless soils are very dry. R_{SPAC} was calculated from pre-dawn and mid-day measurements of plant moisture stress and transpirational flux density (after log transformation) as described in Appendix C.

The experiment was a completely randomized design with a factorial arrangement of inoculation and temperature treatments and 24 seedlings in each treatment combination. The data were tested by analysis of variance (ANOVA) using SYSTAT [97]. A Bonferroni multiple comparison method [68] was used to compare treatments.

RESULTS

Resistance to water flow, R_{SPAC}

There were significant differences in R_{SPAC} among inoculation treatments three weeks after transplanting, at the 6°C soil temperature ($p = 0.05$), but not at the 12°C soil temperature (Table 5). At 6°C, values of R_{SPAC} 21 days after transplanting were greatest for seedlings inoculated with E-strain or L. bicolor, intermediate for those inoculated with H. crustuliniforme, and least for those inoculated with forest floor, T. terrestris or sterilized agar (control).

Table 5. The relationship of R_{SPAC} to inoculum and soil temperature 21 days after transplanting (absolute value of R_{SPAC}, n=8, standard error of estimate in brackets).

	R_{SPAC} [MPa/log$_e$(ugcm^{-2}s^{-1})]	
Soil temperature	6°C	12°C
Control	0.011 (0.007)	0.016 (0.009)
E-strain	0.053 (0.004)	0.012 (0.009)
Forest Floor	0.016 (0.006)	0.011 (0.005)
H. crustuliniforme	0.032 (0.008)	0.006 (0.004)
L. bicolor	0.060 (0.014)	0.020 (0.010)
T. terrestris	0.008 (0.006)	0.009 (0.005)

Mycorrhizal infection has the potential to alter five parameters [38] affecting plant resistance to water flow, or its inverse hydraulic conductivity:

(1) total size, volume or surface area of the root system [16],

(2) plant size and shoot-to-root ratio [45] [52],

(3) new root growth or proportion of unsuberized roots [47] [83],

(4) nitrogen and phosphorus nutrition [34] [76] [81],

(5) and root disease [56].

Correlation analysis was used to examine the relationship of R_{SPAC} to these five parameters. R_{SPAC} was not correlated with new root growth (from the time of transplanting to 21 days), total seedling dry weight nor shoot-to-root ratio at either temperature. Symptoms of root disease (e.g., necrosis) were not observed on any seedlings. R_{SPAC}, however, was correlated with root biomass and nutrient content at the time of transplanting.

At the 6°C soil temperature, R_{SPAC} 21 days after transplanting was negatively correlated with lateral root length ($r = -0.90$, $p = 0.01$) and number of short roots per seedling ($r = -0.90$, $p = 0.01$). Inoculation influenced these root parameters ($p = 0.003$). Seedlings inoculated with E-strain or L. bicolor, which had the highest resistance to water uptake (R_{SPAC}) values 21 days after transplanting, had approximately 50% fewer short roots and 50% less lateral root length per seedling at the time of transplanting compared to control seedlings or those inoculated with other fungi.

A similar result was reported by Coleman et al. [19]. They found that mycorrhizal inoculation increased root resistance to water flow in Douglas-fir seedlings. This effect was independent of P nutrition. Coleman (pers. comm.)

concluded that the smaller size (dry weight) of mycorrhizal root systems (compared to nonmycorrhizal control seedlings) was the major factor responsible for the difference in root resistance.

At the 12°C soil temperature, R_{SPAC} was not correlated with lateral root length or with the number of short roots per seedling ($p > 0.50$). R_{SPAC}, however, was negatively correlated with root nitrogen content ($r = -0.91$, $p = 0.01$) and with root phosphorus content ($r = -0.84$, $p = 0.04$) at the time of planting. Seedlings inoculated with L. bicolor had the lowest root N and P content and the highest value of R_{SPAC} at 12°C. N and P deficiencies have been shown to increase root resistance to water flow [75] [76]. The mechanism involved is not known; however, P deficiencies are known to alter membrane permeability [77].

Pre-dawn Stomatal Conductance

Inoculation had no effect on pre-dawn conductance during the experiment. Pre-dawn stomatal conductance values were very low (averaging less than 0.04 cms^{-1}) indicating that stomata were closing at night. Two days after transplanting, pre-dawn conductance was reduced ($p = 0.05$) at 6°C soil temperature (0.02 cms^{-1}) compared to a conductance of 0.04 cms^{-1} at 12°C. Temperature had no effect on conductance 9 and 21 days after transplanting.

The cold storage effect on pre-dawn stomatal conductance reported by Grossnickle and Blake [35] (i.e., cold-stored seedlings were not able to fully close their stomata) was not observed in this study, possibly because the storage period was much shorter in this study (2.5 months) compared to theirs (6 months).

It would be useful to know if inoculation has an effect on stomatal conductance when seedlings are stored for longer periods. VAM infections have

been shown to influence stomatal conductance [51], possibly by altering the balance of plant growth regulators (e.g., cytokinins or abscisic acid) affecting stomatal opening and closing.

Net Photosynthesis

Inoculation influenced net photosynthesis (Pn) during the experiment. However, Pn was not affected by soil temperature or by interactions between the temperature and inoculation treatments. Twenty-one days after transplanting, Pn rate was higher ($p = 0.01$) in control, forest floor and L. bicolor treatments compared to E-strain, H. crustuliniforme and T. terrestris treatments (Table 6).

Pn 21 days after transplanting was correlated positively with shoot N concentration (% oven-dry weight) ($r = 0.78$, $p = 0.003$), total seedling N ($r = 0.66$, $p = 0.02$) and ratio of shoot N to seedling N ($r = 0.70$, $p = 0.01$). Values of Pn correlated to a lesser degree with shoot P% ($r = 0.50$, $p = 0.10$) and the ratio of shoot P to seedling P ($r = 0.57$, $p = 0.05$). Numerous studies have shown that plant N and P nutrition affect Pn [56]. The Pn rate of Douglas-fir needles, for example, increases with increasing foliar N concentration up to an optimum level of 1.74% [12].

Inoculation significantly influenced ($p = 0.01$) shoot N%, total seedling N, and N allocation between the root and shoot (Table 6). The latter effect was important for the E-strain treatment. Total nitrogen uptake by E-strain seedlings was similar to that by control, forest floor and L. bicolor seedlings. However, less of this N was located in the shoot in E-strain seedlings, resulting in a lower levels of shoot N and Pn compared to seedlings in control, forest floor and L. bicolor treatments.

Mycorrhizal infection can potentially influence Pn by improving the nutrient and water status of seedlings or by increasing the demand for carbohydrates. In this experiment, inoculation effects on Pn were likely related to changes in N and P nutrition. The carbon demand of the various mycorrhizal root systems was not measured. However, since soil temperature had no effect on Pn, even though root growth was 2-fold greater at the higher soil temperature; it is unlikely that differences in carbon demand were responsible for the inoculation effect on Pn.

There was no correlation ($r = -0.21$, $p = 0.13$) between Pn and plant moisture status (U_{xylem}). Pn of conifer seedlings is relatively constant over a wide range of xylem pressure potentials (U_{xylem}). Beadle et al. [7] found Pn of Sitka spruce decreased only when U_{xylem} reached -2.0 MPa. The lowest value of U_{xylem} measured in this experiment was -1.6 MPa, suggesting water stress did not limit Pn.

Table 6. Comparison of net photosynthesis 21 days after transplanting with nitrogen nutrition of spruce seedlings at the time of planting.

Inoculation treatment	Net PS * umoles m^{-2} s^{-1}	Shoot N % oven dry wt.	Plant N mg	S:P** N ratio
n	10	2	2	2
Forest Floor	1.4	1.3 a ***	4.7 a	0.7 a
Control	1.3	1.2 ab	3.7 a	0.7 a
L. bicolor	1.2	1.1 b	3.8 a	0.8 a
T. terrestris	1.0	0.8 c	3.1 ab	0.6 ab
E-strain	0.9	0.9 bc	3.6 a	0.5 b
H. crustuliniforme	0.8	0.9 bc	3.2 ab	0.6 ab

* Log transformation of Pn was used for analysis of variance.

** S:P N ratio is the ratio of shoot N content to total plant N content.

*** Column means followed by the same letter are not significantly different ($p = 0.01$).

SECTION FOUR

OBJECTIVE

Compare the relative ability of various fungal symbionts to improve seedling growth and nutrient uptake at cool soil temperatures.

BACKGROUND INFORMATION

There is considerable evidence that mycorrhizal infection can increase the nutrient uptake of host plants either (1) by increasing the nutrient absorbing surface of the root system, or (2) by increasing the efficiency of uptake (e.g., utilizing organically complexed or insoluble nutrients not normally available to plants) or (3) by increasing the longevity of short roots [38]. ECM inoculation may increase the growth of white spruce planted into cold soils if nutrient availability is limiting growth. Fertilizer application has been shown to stimulate the growth of horticultural plants growing in cold soils [20] [73]. Foliar analyses conducted by Ballard [5] indicate that nitrogen, copper and iron may be deficient in many interior spruce plantations.

The ability of mycorrhizal seedlings to absorb nutrients depends on the temperature optimum for the fungal symbiont. Research with vesicular-arbuscular mycorrhizae (VAM) has shown that cool temperatures eliminate the benefits normally derived from mycorrhizal infection [65] [87]. The growth of VAM plants may even be reduced compared to that of nonmycorrhizal plants at low temperatures [32] [39] [84]. These negative effects may be due the inability of established ECM fungi to absorb or transport phosphorus while still utilizing host carbon. Smith and Roncadori [87] reported that mycorrhizae increased the phosphorus and copper uptake of cotton plants at

soil temperatures of 24°C and 36°C; but mycorrhizae had no effect on nutrient uptake at a soil temperature of 18°C.

METHOD

The experiment was a completely randomized design with a factorial arrangement of seven inoculation treatments and two soil temperatures; 24 seedlings per treatment combination. The inoculation treatments included: (1) a control (sterilized agar medium), (2) forest floor inoculum, and pure cultures of (3) Hebeloma crustuliniforme 5249, (4) Laccaria bicolor 5268, (5) Amphinema byssoides 0288, (6) Thelephora terrestris 2088, (7) E-strain 947. Except for the Amphinema and Thelephora isolates, the test inocula were obtained from northern B.C. or Alberta forest sites (Appendix A).

Seedlings were inoculated and grown as described on page 11. After cold-storage, they were planted into a mixture of forest floor, mineral soil and perlite (1:1:2 by volume) at two soil temperatures: 6 and 12°C. They were then grown for 12 weeks in a controlled environment (18 hr photoperiod, relative humidity of 30 to 50%, air temperature of 12 to 20°C, and a light intensity of 400 uE $m^{-2}s^{-1}$.)

Growth, nitrogen and phosphorous nutrition of the seedlings were measured at the start of the experiment; then five and twelve weeks later. Nutrient sampling and analysis were conducted as described on page 17. Growth measurements included: biomass of root, mature shoot and current foliage, lateral root length, short root number and caliper. Data were tested with ANOVA or analysis of covariance using initial caliper as the covariate.

Efficiency of N and P uptake was calculated per unit root dry weight-day (analogous to person-day as defined by [41]) assuming a linear relationship between root growth and time. This estimate of nutrient uptake is independent

of plant size and shoot-to-root ratio. Percent caliper growth was calculated as:

$$\% \text{ caliper growth} = \frac{(\text{final caliper} - \text{initial caliper}) * 100}{(\text{initial caliper})}$$

RESULTS

Shoot growth

Percent caliper growth responded to soil temperature ($p < 0.001$) and inoculation treatments ($p < 0.001$); no interaction occurred between these main effects. Caliper growth was similar for uninoculated and inoculated seedlings (when all six inoculation treatments were combined for comparison with the control). However, there was considerable difference in caliper growth among inoculation treatments (Table 7). Seedlings infected with forest floor, *L. bicolor* or *T. terrestris* grew more in caliper ($p = 0.003$) than did control seedlings or seedlings infected with the other fungal isolates.

Inoculation treatment, but not soil temperature, influenced current foliage biomass (CFB). Current foliage biomass, like caliper growth, varied considerably among inoculation treatments (Table 7). Seedlings inoculated with forest floor and *L. bicolor* had more CFB than did control seedlings or seedlings inoculated with the other fungal isolates ($p < 0.001$).

Root growth

Inoculation influenced ($p < 0.01$) root biomass at the 12°C soil temperature; but had no effect ($p = 0.18$) at 6°C (Table 7). At 12°C, E-strain, *H. crustuliniforme* and *A. byssoides* inoculation resulted in lower root

biomass ($p = 0.001$) compared to other treatments (including the control). New root and shoot growth were positively correlated ($r = 0.86$, $p = 0.012$) at the 12°C but not the 6°C soil temperature. At 6°C, the ratio of current foliage produced per unit of new root biomass was twice that at 12°C; this resulted in a higher shoot-to-root ratio at the lower soil temperature for all inoculation treatments.

Effects of Inoculation on Seedling Growth

Regardless of soil temperature, seedlings inoculated with forest floor or L. bicolor had the greatest growth; seedlings inoculated with E-strain or H. crustuliniforme had the poorest growth; control seedlings and those inoculated with A. byssoides or T. terrestris had an intermediate level of growth.

The poor performance of seedlings infected with E-strain or H. crustuliniforme was not related to weak mycorrhiza infection or to the source of the inoculum i.e., field or nursery. At the start of the experiment, more than 95% of the short roots of these seedlings were mycorrhizal with the inoculant fungus. The least and most effective inocula tested in this study were from field sources; the two nursery isolates, A. byssoides and T. terrestris were intermediate in effectiveness.

Seedling nutrition

Very marked differences were found in current foliage N and P nutrition (Table 8). N and P concentrations (% oven-dry weight) and contents (mg/seedling) were consistently higher in seedlings grown at 12°C compared to those grown at 6°C. Foliar N and P concentrations were compared to literature values reported for spruce [6] [8] [9] [31] [44] [67] [89]. In comparison to

these studies: (1) foliar N levels of seedlings grown at 6°C were low to very severely deficient; (2) foliar N levels of seedlings grown at 12°C were low to adequate, and (3) foliar P levels were adequate to optimum at both soil temperatures.

Because of differences in growth, concentration data were a misleading indicator of nutrient content or uptake for some inoculation treatments. At 12°C soil temperature, for example, current foliage N% was highest with E-strain inoculation; however, N content of current foliage was low compared with other treatments (Table 8).

Table 7. Effect of inoculation on initial caliper, final caliper (at 12 weeks), caliper growth during the experiment, and current foliage biomass (CFB) and root biomass (oven-dry wt.) at 12 weeks.

	Initial caliper	Final caliper	Caliper* growth	CFB	Root biomass	
	------mm----------		-(%)-	-mg-	----mg-----	
					6°C	12°C
n	48	16	16	16	8	8
Control	1.4 (0.2)	2.1 (0.3)**	56	150	220	260
Forest floor	1.3 (0.2)	2.2 (0.4)	79	278	190	300
E-strain	1.2 (0.2)	1.7 (0.4)	53	98	200	230
L. bicolor	1.1 (0.2)	2.0 (0.3)	82	244	180	290
H. crustuliniforme	1.2 (0.2)	1.7 (0.3)	46	113	170	190
A. byssoides	1.2 (0.2)	1.9 (0.4)	54	169	200	240
T. terrestris	1.2 (0.1)	2.0 (0.3)	71	151	200	280

* Adjusted means; log transformation was used for covariance analysis of CFB.

** Standard deviation in brackets.

Inoculation treatments were ranked from highest to lowest on the basis of N and P content in current foliage at both soil temperatures. Ranking of the treatments was similar for both nutrients at 6°C and 12°C: (1) highest values for forest floor and L. bicolor, (2) intermediate values for A. byssoides, T. terrestris, and control, (3) lowest values for E-strain and H. crustuliniforme.

Table 8. Effect of soil temperature and inoculation treatment on N and P nutrition of current foliage.

Inoculation treatment	Temp °C	CFB mg	N%* -oven-dry wt-	P%	N -mg/seedling-	P
Control	6	126	0.65	0.17	0.82	0.21
	12	172	0.87	0.22	1.50	0.38
Forest floor	6	244	0.62	0.15	1.51	0.37
	12	323	1.03	0.21	3.33	0.68
E-strain	6	109	0.51	0.13	0.56	0.14
	12	94	1.67	0.22	1.57	0.21
L. bicolor	6	225	0.66	0.20	1.49	0.45
	12	284	0.93	0.26	2.64	0.74
H. crustuliniforme	6	131	0.47	0.14	0.62	0.18
	12	101	0.93	0.20	0.94	0.20
A. byssoides	6	139	0.82	0.19	1.14	0.26
	12	214	1.04	0.23	2.22	0.49
T. terrestris	6	154	0.75	0.18	1.16	0.28
	12	157	1.23	0.25	1.93	0.39

* Based on a bulk sample of eight seedlings per treatment combination.

The poor performance of seedlings infected with E-strain or H. crustuliniforme was likely a result of the poorer nutrition of these seedlings. Growth parameters correlated with total seedling N and P content measured at the beginning of the experiment; and with N and P uptake during the experiment (Table 9). With the exception of E-strain seedlings at the 12°C soil temperature, seedlings inoculated with E-strain or H. crustuliniforme had lower N and P uptake during the experiment compared to control seedlings or seedlings inoculated with other fungi. Low rates of nutrient uptake were correlated with poor root growth (Table 9) and in the case of E-strain infected seedlings with a low efficiency of nutrient uptake in 6°C soil (Figure 1).

Table 9. Pearson correlation coefficients (r) for seedling growth and nutrition (n = 14).

N and P foliage nutrition	Caliper growth	Root growth	Current biomass
Initial content			
N*	0.82 (<0.01)**	0.60 (0.02)	0.77 (0.00)
P*	0.79 (<0.01)	0.56 (0.04)	0.65 (0.01)
Uptake during experiment			
N*	0.73 (<0.01)	0.78 (<0.01)	0.35 (0.21)
P*	0.80 (<0.01)	0.90 (<0.01)	0.60 (0.02)

* mg/seedling.
** Probability level in brackets.

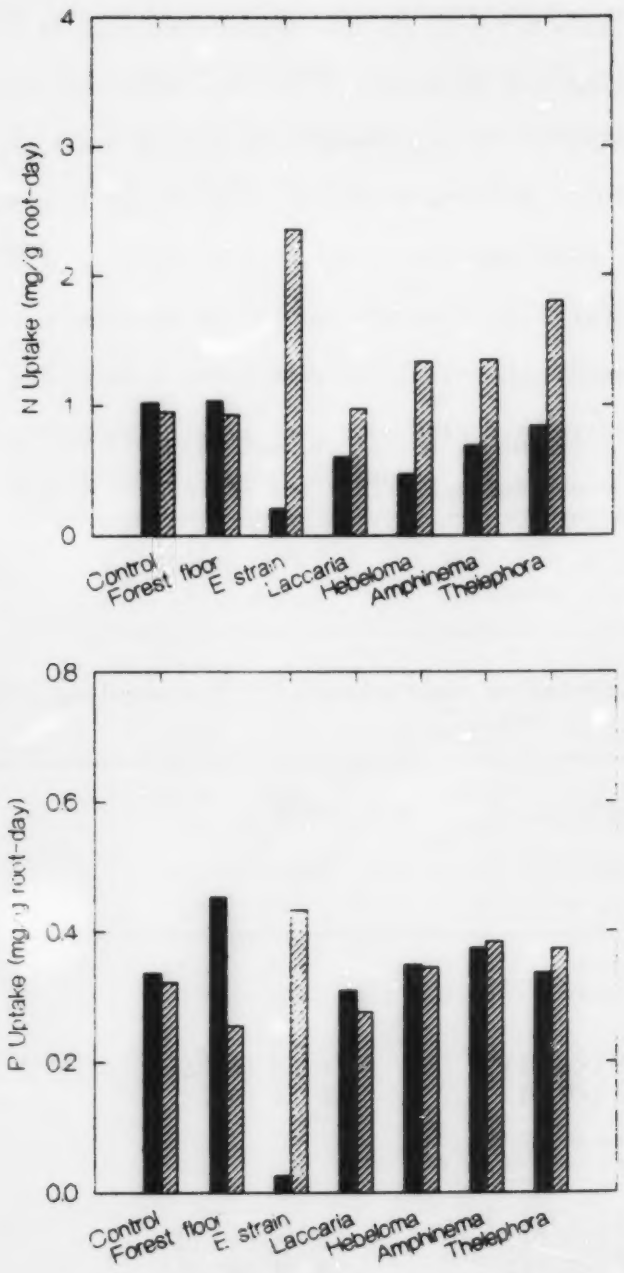

Figure 1. **N and P uptake ($\mu g g^{-1}$ root-day) by inoculation treatment and soil temperature: dark bars - 6°C; light bars - 12°C.**

SECTION FIVE

OBJECTIVE

Examine the effect of a slow-release fertilizer applied at the time of planting on persistence of inoculant fungi, host plant growth and nutrient uptake.

BACKGROUND INFORMATION

Applying slow-release fertilizers at the time of planting is one silvicultural option for improving early seedling growth. However, high rates of fertilization, particularly of nitrogen, have been shown to inhibit mycorrhizal formation in nurseries [21] [43] and in forest stands [33] [74] [90]. ECM fungi vary in their response to fertilizer [2] [43]. Hunt [43] found that T. terrestris formed mycorrhizae readily in growing mixes with high nutrient levels; E-strain did not. Two studies testing both artificial inoculation and fertilizer at the time of planting [36] [61] are not applicable to backlog reforestation sites in northern B.C. It would be useful to know if fertilization at the time of planting influences the persistence of established mycorrhizae or alters their effects on seedling growth and nutrition.

METHOD

Seedlings inoculated with forest floor, E-strain 947 or H. crustuliniforme 5249 and grown as described on page 11 were transplanted into either an unfertilized or fertilized mixture of forest floor, mineral soil and perlite (1:1:2 by volume). The forest floor mixture was fertilized by adding

a slow-release fertilizer Osmocote 14-14-14 (Sierra Chemical Co., Milpitas, California) at the rate of 100 kg N/ha.

All seedlings were grown at a 12°C soil temperature in a controlled environment chamber. Nutrition and growth were assessed (as described in sections three and four) at the time of transplanting, then five and twelve weeks later. The experiment was a completely randomized 3 x 2 x 3 factorial (3 fungal isolates, 2 fertilizer regimes, 3 sample times) with eight seedlings per treatment combination. Data were analysed as described in sections two and four.

RESULTS

Persistence of inoculant fungi

At the time of transplanting, mycorrhiza class averaged 6.0, 5.9, and 6.0 respectively for the E-strain, H. crustuliniforme and forest floor inocula. In the unfertilized mix, colonization of new root growth outside the original plug was greater than 75% for all inoculant fungi. Fertilization reduced ($p = 0.05$) the colonization of new short roots by E-strain by 10 to 25%; other short roots were colonized by forest floor fungi. Fertilization had no effect on the persistence of forest floor or H. crustuliniforme inocula.

Seedling Growth

Caliper growth, current foliage biomass and root dry weight were affected by inoculation ($p = 0.001$) and fertilizer ($p = 0.001$) treatments. There was no interaction between the effects of fertilizer and inoculation on seedling growth. In both fertilized and unfertilized soils, seedlings

inoculated with forest floor had greater caliper growth, current foliage and root biomass at 12 weeks ($p < 0.001$) (Table 10) than did seedlings inoculated with either H. crustuliniforme or E-strain fungi.

Table 10. Effect of slow-release fertilizer and three inocula on percent caliper growth, root biomass (oven-dry wt.) and current foliage (CF) biomass (oven-dry wt.) at 12 weeks; n=8.

	- NPK			+ NPK		
	caliper growth	root* -biomass-	CF*	caliper growth	root --biomass--	CF
	---%---	----mg---		---%--	---mg------	
Forest floor	104	289	323	193	481	784
E-strain	52	203	94	128	286	332
H. crustuliniforme	48	175	101	128	342	367

* Adjusted means; log transformation used in covariance analysis of CFB and root biomass data.

Seedling Nutrition

Fertilization had minor effects on foliar N and P levels with two exceptions: (1) it increased foliar N from low to adequate levels in the forest floor treatment and (2) it decreased foliar P in the H. crustuliniforme treatment (Table 11).

Total N and P uptake during the experiment was increased by fertilization and was greatest for forest floor seedlings. Total N and P uptake was not correlated ($p = 0.25$) with efficiency of N and P uptake i.e.,

uptake per unit root dry weight, but was highly correlated with new root growth ($r = 0.94$, $p = 0.005$). This result suggests that fungal effects on nutrient uptake resulted more from changes in size of the nutrient absorbing system rather than from changes in the efficiency of nutrient uptake on a unit root basis.

Table 11. Effect of slow-release fertilizer and inoculation treatments on N and P nutrition of current foliage at 12 weeks.

Inoculation treatment	N	P	N	P
	% oven-dry wt.		mg/seedling	
Forest floor				
- NPK	1.03	0.21	3.33	0.68
+ NPK	1.73	0.19	13.56	1.48.
E-strain				
-NPK	1.67	0.22	1.57	0.21
+NPK	1.80	0.19	5.98	0.63
H. crustuliniforme				
-NPK	0.93	0.20	0.94	0.20
+NPK	0.90	0.15	3.30	0.55

GENERAL DISCUSSION

The objective of FRDA 52-41-109 was to study the influence of soil temperature on the effectiveness of ectomycorrhizal (ECM) fungi as defined by [94], i.e., the ability of ECM fungi (1) to colonize roots in conifer nurseries, (2) to persist in the field, and (3) to improve seedling growth and physiology. The following section briefly summarizes the results of this study and discusses their implications for research and reforestation programs.

Soil Temperature and Mycorrhiza Formation

Soil temperature influences mycorrhizal colonization in container nurseries. In Experiment No. 1, root colonization by 14 fungal isolates was compared at three soil temperatures (6, 16, 26 °C). Seedlings were grown in Spencer-Lemaire Rootrainers filled with a peat:vermiculite medium.

On average, fungi collected from cooler forest sites formed mycorrhiza at all growing mix temperatures more rapidly than did nursery isolates or isolates collected from warmer forest sites. However, there was considerable variability among fungi within each source. Some, but not all, field isolates formed mycorrhiza relatively well at 26°C; some, but not all, nursery isolates formed mycorrhiza relatively well at 6°C.

Soil Temperature and Persistence of Established Mycorrhizal Infections

After planting, the degree of mycorrhiza formation by an inoculant fungi on new roots is determined by its competitiveness with the indigenous microflora and its adaptation to a particular soil environment [94]. In Experiment No. 2, the effect of cool soil temperatures (6 ,12°C) on

persistence (i.e., colonization of new roots after transplanting) by inoculant fungi was studied in forest soils with and without indigenous ECM fungal propagules.

Overall, persistence of inoculant fungi on new roots was high (> 75%) three months after transplanting, even in the forest soil mixture containing ECM fungal propagules. Soil temperature had no effect on persistence in the absence of ECM fungal propagules. When ECM propagules were present, persistence was lower at 12°C compared to 6°C. Most short roots not colonized by the inoculant fungi were colonized by forest floor fungi. Because of the greater root growth at 12°C, new short roots were more likely to contact indigenous ECM propagules at this soil temperature.

Experiment No. 2 was conducted in relatively infertile soils. Applying slow-release fertilizers at the time of planting is one silvicultural option for improving seedling growth. Fertilizer has been shown to inhibit mycorrhizal formation in nurseries [21] [43] and in forest stands [33] [74] [90]; but there have been few studies of the effect of fertilizer at the time of planting on persistence of inoculant fungi. Therefore, the effect of slow-release fertilizer on persistence of three inocula (E-strain, H. crustuliniforme, and forest floor) was tested in Experiment No. 5. Slow-release fertilizer reduced the persistence of the E-strain but had no effect on the persistence of the forest floor or H. crustuliniforme inocula.

Effect of Mycorrhizal Inoculation on Spruce Seedling Growth and Physiology

Physiology

In experiment No. 3, the ability of uninoculated newly transplanted seedlings to acclimate to cool soil temperatures (6, 12°C) was compared to

that of seedling inoculated with various ECM fungi. Three physiological parameters were used to measure acclimation: resistance to water uptake (R_{SPAC}), net photosynthesis (Pn), and pre-dawn stomatal conductance. Inoculation treatments influenced Pn and R_{SPAC} but not pre-dawn stomatal conductance. Pn and R_{SPAC} were significantly correlated with the initial size and nutrient status of spruce seedlings; these parameters were altered significantly (both positively and negatively) by inoculation in the nursery .

Growth and nutrient uptake

In Experiment No. 4, white spruce seedling nutrient uptake and growth was studied for three months in forest soils at two temperatures (6°C or 12°C). The seedlings were inoculated with either forest soil, E-strain 947, Hebeloma crustuliniforme 5249, Laccaria bicolor 5268, Thelephora terrestris 2088, Amphinema byssoides 0288 or autoclaved agar (control seedlings). No significant interactions occurred between soil temperature and inoculation. Regardless of soil temperature, seedlings inoculated with forest soil or Laccaria bicolor 5268 had the greatest growth and nutrient uptake. Caliper growth of seedlings infected with these inocula was 40 to 45% greater than that of control seedlings; current foliage biomass was 63 to 85% greater.

Inoculation with E-strain and H. crustuliniforme under the conditions of this study (cool, wet soils with indigenous ECM fungi) reduced seedling nutrient uptake and growth compared to uninoculated seedlings. Caliper growth was reduced by 5 to 25%; current foliage biomass by 25 to 35%.

Implications for Reforestation Programs

(1) This controlled environment study suggests artificial inoculation of spruce seedlings, with forest soil inoculum or specific ECM fungi, has good potential to improve field performance in cool (6-12°C), wet soils at least in the first year after outplanting. However, field research must be conducted before mycorrhizal benefits can be predicted with confidence.

Of the fungal species tested, L. bicolor is a particularly promising ECM fungus for artificial inoculation because its basic physiology and genetics are being researched at the Université Laval [49]. This information is essential if the long-term goal of artificial inoculation programs (i.e., the breeding of superior strains of ECM fungi), is to be realized.

(2) The study also suggests that some ECM fungal isolates may reduce seedling nutrient uptake and growth in the first year after planting. Therefore, it is essential to investigate further the effects of ECM fungi (e.g., T. terrestris and E-strain) which naturally infect spruce seedlings in the nurseries, on the field performance of seedlings planted in cool, wet soils. The results of this study do not support the hypothesis proposed by Marx [58] that any ECM fungus on a nursery seedling is better than no ECM fungus. Two inocula (L. bicolor, forest floor) increased growth relative to control seedlings; two inocula (A. byssoides, T. terrestris) had no effect; and two inocula (E-strain, H. crustuliniforme) decreased growth.

(3) Forest soil inoculum should be considered as an alternative to pure culture inoculum, if funding and technology is not available to investigate the effectiveness of specific fungi. Seedlings inoculated with forest soil

may benefit from the greater diversity of ECM fungal propagules and rhizosphere organisms contained in soil inoculum compared to pure culture inoculum [72]. However, there are several disadvantages to forest soil inoculation: (a) the need to handle large volumes of forest soil, (b) the risk of introducing soil-borne pathogens into container nurseries and (c) the lack of control over the specific fungi forming mycorrhiza.

(4) Temperature effects on mycorrhiza formation have at least two implications for artificial inoculation programs. Will it be possible to successfully inoculate seedling growing in warm container mixes with isolates indigenous to cool soil environments? Will mycorrhizal infections established in a warm nursery environment be able to persist and colonize roots formed after the seedlings are planted into cool soils? Based on the fungal isolates used in this study, the answer to both question is "yes". However, growing mix temperature likely plays an important role in competition between inoculant fungi and indigenous nursery fungi in container nurseries. T. terrestris, an indigenous nursery fungus, which often contaminates artificial inoculation experiments, had a higher rate of mycorrhizae formation at 26°C compared to many of the other isolates. This could give it an advantage in container nursery medium with high growing mix temperatures.

(5) It is well known that there are significant interactions between host plants, ECM fungi, and the environment of the planting site [94]. The slow-release fertilizer experiment points to the need to select ECM fungi that also interact favorably with silvicultural regimes.

Implications for Research

(1) Soil temperature alters mycorrhizal effectiveness. Therefore, soil temperature should be recorded in both field and controlled environment studies in order to better interpret and compare results with other studies. In general, a better understanding of the influence of the planting environment on mycorrhizal effectiveness will help unravel the contradictory results of artificial inoculation studies.

(2) In order to properly evaluate the potential of artificial inoculation for cool forest sites, a culture collection of ECM fungi indigenous to northern B.C. forest sites is essential. Many fungal species and isolates within species need to be tested before the benefits of inoculation can be assessed for a particular forest site, conifer species and silvicultural regime.

The availability of ECM fungal isolates limited the scope of this study to one or two isolates of each fungal species. It was not possible, for example, to obtain an T. terrestris isolate from a northern forest. An isolate indigenous to northern forests would likely have been more effective in this study than was the nursery isolate tested. Holden et al. [42] reported that Sitka spruce seedlings inoculated with a forest isolate of T. terrestris were significantly larger than those with a nursery isolate when the seedlings were grown in forest soil.

(3) Research should be directed to examining the effects of the planting site environment and forest management practices (e.g., mechanical site preparation) on indigenous ECM fungal inoculum in order to identify sites and silvicultural regimes most likely to benefit from artificial inoculation. If the greenhouse bioassay method is used to assess inoculum potential, soil temperatures should be maintained at values characteristic of the planting site.

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APPENDIX A

SOURCE OF FUNGAL ISOLATES

Amphinema byssoides (Fr.) J. Erikss. (0188)

Author. Isolated in 1988 from surface-sterilized ectomycorrhizae of container spruce seedlings grown at the Balco Canfor Reforestation Centre, Kamloops, B.C.

E-strain (sensu Mikola) (947)

Dr. R.M. Danielson's collection at the University of Calgary. Isolated in 1978 from white spruce seedlings growing in a fertilized subalpine soil.

E-strain (0188)

Author. Isolated in 1988 from surface-sterilized ectomycorrhizae of container Douglas-fir seedlings grown at the Harrop Nursery, B.C.

Forest Floor

Author. Bulked sample collected in 1987 from three juvenile spruce plantations near Mackenzie, B.C.

Hebeloma crustuliniforme (Bull. ex St. Amans) Quelet (5247,5249)
University of Alberta, Microfungus Collection. Collected in 1985 from Picea glauca woods, Lac La Biche forest.

Hebeloma crustuliniforme (Bull. ex St. Amans) (5)
University of Washington Collection (Dr. Bledsoe). Isolated in 1984 from a mixed conifer forest, Wenatchee National Forest (550m).

Hebeloma crustuliniforme (Bull. ex St. Amans) (8)
University of Washington Collection (Dr. Bledsoe). Isolated in 1971 from a Douglas-fir forest, Benton County, Oregon.

Hebeloma crustuliniforme (Bull. ex St. Amans) (125)
From Dr. G. Hunt, Balco Canfor Reforestation Centre, Kamloops, B.C. Isolated in 1985 from a mixed spruce, fir and cedar forest near Barrier Lake, B.C. (1400m).

Laccaria bicolor (R. Mre.) Orton (5268)

University of Alberta Microfungus Collection. Isolated in 1985 from a pine-spruce forest, Slave Lake Forest, Alberta.

Laccaria bicolor (R. Mre.) Orton (5313)

University of Alberta Microfungus Collection. Isolated in 1976 from Larch container seedlings in FSL greenhouses, Corvallis, Oregon.

Laccaria laccata (Scop. ex Fr.) Berk.& Br. (101C,D)

From Dr. G. Hunt, Balco Canfor Reforestation Centre, Kamloops, B.C. Isolated from sporocarps in the nursery.

Thelephora terrestris Ehrhart ex Fr. (2088)

Author. Isolated in 1988 from surface-sterilized ectomycorrhizae of spruce seedlings grown at CIP nursery, Saanichton, B.C.

Thelephora terrestris Ehrhart ex Fr.(Laval)

University of Laval CRBF Culture Collection. Isolated in 1980 from a young poplar stand near Quebec City.

APPENDIX B

INITIAL MYCORRHIZAL STATUS

Overall colonization by inoculant fungi was very good (>75%) at the start of experiments three, four and five. Table B1 shows the degree of mycorrhizal formation by inoculant and contaminant fungi for all three experiments.

(1) Control seedlings:

60% were nonmycorrhizal; the remainder had a light infection of Thelephora terrestris (mantle development was weak and external hyphae and mycelial strands were rare).

(2) Seedlings inoculated with Hebeloma crustuliniforme (5249), E-strain (947) and Laccaria bicolor (5268), Thelephora terrestris (2088):

100% with inoculant fungus forming mycorrhizae on more than 50% of the short roots; other short roots were mainly non-mycorrhizal.

(3) Seedlings inoculated Thelephora terrestris (Laval)

72% with more than 50% of the short roots were colonized by the inoculant fungus. These Thelephora mycorrhizae were well-developed (i.e., mantles covered more than 75% of the short roots, cystidia and mycelial strands were common) strongly suggesting that they developed from the pure culture inoculant fungus and not from a Thelephora nursery contaminant. Non-Thelephora short roots were either non-mycorrhizal or colonized by E-strain fungi.

(4) Seedlings inoculated with forest floor

85% were colonized primarily by forest floor fungi; 15% had 50% or more of the short roots colonized weakly by Thelephora terrestris.

(5) Seedlings inoculated with Amphinema byssoides

90% with inoculant on more than 50% of short roots; light Thelephora terrestris infection.

Table B1. Percentage of seedlings in each of six mycorrhiza classes

Inoculation treatment	Exp	Mycorrhiza class *					
		1	2	3	4	5	6
Control	3,4						
C **		60	20	10	4	6	0
E-strain 947	3-5						
I		0	0	0	0	6	94
Hebeloma crustuliniforme 5249	3-5						
I		0	0	0	0	5	87
Laccaria bicolor 5268	3,4						
I		0	0	0	30	50	20
Thelephora terrestris 2088	4						
		0	0	0	0	20	80
Thelephora terrestris (Laval)	3						
I		0	20	8	16	32	24
C		70	12	12	4	2	0
Forest Floor	3-5						
I		0	5	10	0	12	73
C		73	12	0	15	0	0
Amphinema byssoides 0288	4						
I		0	0	10	15	75	0
C		75	25	0	0	0	0

* Classes: (1) 0% mycorrhiza (2) 1-25% (3) 26-50%
(4) 51-75% (5) 75-95% (6) >95%

** C=contaminant fungi, I=inoculant fungi.

APPENDIX C

R_{SPAC} ESTIMATION

R_{SPAC} was measured indirectly using Elfving et al.'s [30] model of plant water relations which is leaf water potential equals soil water potential minus R_{SPAC} times transpirational flux density (TFD) or:

$$\Psi_{leaf}-\Psi_{soil}-R_{SPAC}*(TFD).$$

This model assumes:

1) Experimental plants have negligble capacity for water storage.
2) Xylem water potential is representative of leaf water potential.
3) Water potential of the soil is negligble.

The first two assumptions are reasonable for small seedlings. The third was met by maintaining soil water potential above field capacity.

Three seedlings per inoculation-temperature treatment combination were measured to determine pre-dawn Ψ_{xylem}. Five seedlings were measured to determine mid-day (4 hours into the light period) Ψ_{xylem} and transpirational flux density.

R_{SPAC} for each treatment combination was calculated from the slope of the regression of Ψ_{xylem} on the log of transpiration flux density. A log transformation was required to meet the homogeneity of residual assumption for regression analysis. R^2 values for these regressions ranged from 0.60 to 0.90. Equality of regression slopes (R_{SPAC}) was compared using t-tests [68].